भारतीय मानक
Indian Standard

IS 17213 : 2019

वस्त्र रंजक सामग्री — अनार के छिल्के का रंग — पहचान की विधि

Textile Dyestuffs — Punica Dye — Identification

ICS 59.040

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October 2019 **Price Group 5**

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards after the draft finalized by Textile Speciality Chemicals and Dyestuffs Sectional Committee had been approved by the Textile Division Council.

The revival of natural dyes has been for two reasons:

a) Due to the harmful effect of synthetic dyes, globally safer natural dyes are being preferred; and

b) Ban of azo dyes has also brought back natural dyes in limelight. Natural dyes are being used extensively for dyeing of cotton, silk and wool substrate.

Thus, Punica dye (pomegranate, *Anar ka chilka*) has its own characteristics of being a natural dyestuff and its method of use in dyeing and printing these textile substrates. In order to achieve optimum quality with minimum lot to lot variation and variations in dyeing due to same dye of different manufacturers, it is essential to standardize this natural dyestuff. This will also lead to minimize cost benefit ratio and help the user to adjust his dyeing or printing recipes suitably.

Punica granatum produces yellow dyes present in the pericarp of the fruit which has been used for dyeing textiles since ancient times. The botanical name of Punica is known as *Punica granatum.* It belongs to family Punicaceae. The pomegranate, botanical name *Punica granatum*, is a fruit bearing deciduous shrub or small tree that grows between 5 and 8 m tall.

The fruit is typically available in season in the northern hemisphere from September to February, and in the southern hemisphere from March to May. As intact arils or juice, pomegranates are used in baking, cooking, juice blends, meal garnishes, smoothies, and alcoholic beverages, such as cocktails and wine. The pomegranate originated in the region extending from modern day Iran to northern India, and has been cultivated since ancient times throughout the Mediterranean region.

It is for the first time that the standard for natural dyestuff Punica dye (Pomegranate, *Anar ka chilka*) has been formulated. It is mainly for the ease of buyers, sellers and users of this natural dyestuff. Standardization and identification method will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff from the synthetic analogue which is usually used as adulterant for cost modification and colour enhancement purposes.

The composition of the Committee responsible for the formulation of this standard is given in Annex F.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — PUNICA DYE — IDENTIFICATION

1 SCOPE

This standard prescribes test method and identification requirement of dried/ aqueous extract of punica dye and punica dyed textiles. The detection and identification of synthetic punica dye and natural colorant punica dye are conducted using kit method given in Annex A. Confirmatory tests can be carried out through thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) by chromatographic techniques as given in Annex B and C as well as by UV-visible spectroscopic technique as given in Annex D. The method for extraction of natural punica dye from dyed swatches is given in Annex E.

2 REFERENCES

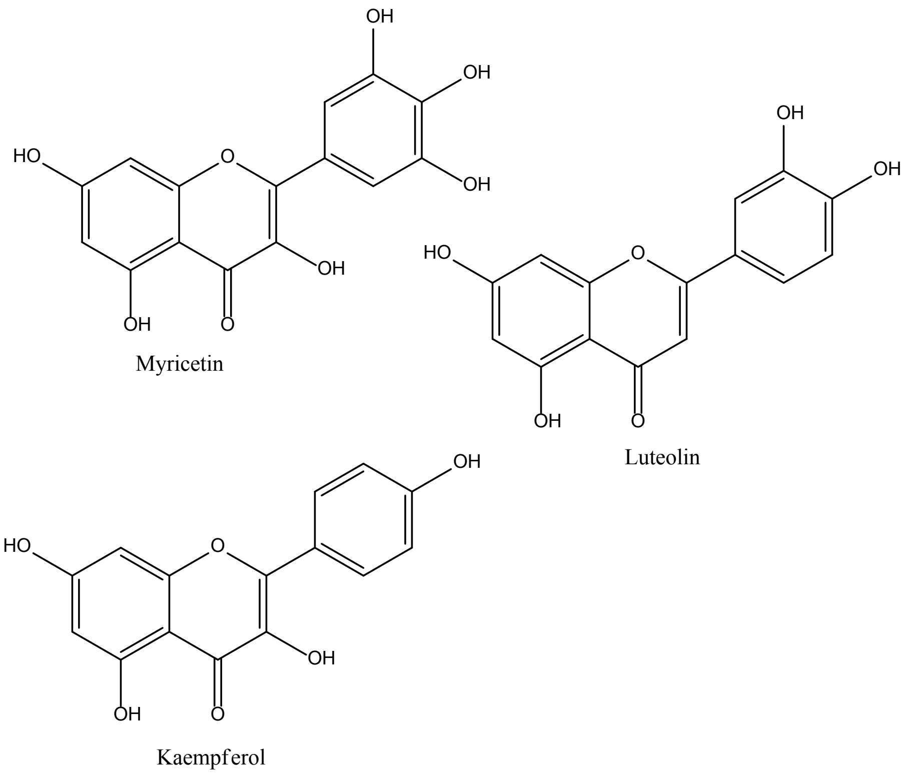
The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
1070 : 1992	Reagent grade water

3 TERMS AND DEFINITIONS

For the purposes of this standard, the following terms and definitions apply:

3.1 Punica — Punica granatum consist of myricetin, quercetin, luteolin and kaempferol in the epicarp of its fruit. The main chemical structures of the natural Punica dye are given below:



Myricetin

Luteolin

Kaempferol

FIG. 1 CHEMICAL STRUCTURES OF THE NATURAL PUNICA DYE

3.2 Natural Colour — Colourants from natural origin, particularly materials obtained from plant parts, stem, root and leaves extracted without any chemical reaction, primarily used in textiles are for dyeing or printing.

4 PRINCIPLE

Natural colourants are usually never a single compound. They are composed of several compounds which are structurally similar, each of them having their characteristic bonding with the fabric based on their structure uniqueness. Their contribution in the total colourant may vary. Therefore, the analysis of these individual components can be carried out by chromatographic and spectroscopic techniques. The authenticity of the natural colourant can be established by these techniques very clearly.

5 PROCEDURE

5.1 Preparation of Natural Punica Dye Sample

Stock solution of natural punica dye sample is prepared by dissolving 100 mg in one litre of methanol. This solution is further used after dilution, if necessary, for chromatographic and spectroscopic analyses.

5.2 Analysis

The detection and identification of natural punica and synthetic yellow dye samples are conducted using kit method given in Annex A, TLC and HPLC by chromatographic techniques as given in Annex B and C as well as by UV-visible spectroscopy as given in Annex D. The method for extraction of natural dye from dyed swatches is given in Annex E.

5.3 Qualification and Identification of Natural Punica

Comparison between analyses of natural punica and synthetic yellow dye through **5.3** could show the results of discrete differences in synthetic and natural punica sample in the kit test. The specific tests to be conducted are given in Table 1.

6 REPORT

The report shall include the following information:

a) Name of the natural dye tested;
b) Test method used; and
c) Identification of the natural source.

7 CHECK OF THE ANALYTICAL SYSTEM

A check of the procedure of analysis and instrument/equipment is also essential. HPLC, UV-visible spectrophotometer must be checked before the analysis of the sample.

8 VALIDATION OF THE METHOD

Method validation and calibration is carried out using authentic and standard natural dye for UV-visible spectrophotometric analysis:

a) Reliability of the method,
b) Repeatability, and
c) Comparability.

Table 1 Specific Tests Recommended for Identification of Natural Punica Dye

(*Clause* 5.3)

Sl No. (1)	Test (2)	Type of Test (3)	Annex No. (4)
i)	Kit test for identification of natural punica dye	Preliminary test	Annex A
ii)	Chromatography tests		
	a) Thin layer chromatography (TLC)	Mandatory test	Annex B
	b) High performance liquid chromatography (HPLC)	Optional test*	Annex C (*see* NOTE)
iii)	Spectroscopy tests		
	a) UV-visible spectroscopy	Confirmatory test	Annex D

NOTE — HPLC may be done instrumentally, wherever facilities are available.

ANNEX A

(*Clauses* 1, 5.2 and 5.3)

KIT TEST FOR IDENTIFICATION OF NATURAL PUNICA DYE

A-1 PRINCIPLE

Each natural dye has its characteristic molecular composition which is the distinctive feature of that dyestuff. Dissolution of the two samples of punica and synthetic yellow dye under the class of yellow dyes in different polarity solvents would bring out the difference and distinction when compared with synthetic yellow dye. The punica dye (pomergranate, *anar ka chilka)* bearing different contaminants dissolves in water layer better, whereas the synthetic yellow dye dissolves well in dichloromethane solvent.

A-2 APPARATUS

A-2.1 Test Tubes

A-2.2 Test Tube Holder

A-2.3 Electronic Balance

A-2.4 Spatula

A-2.5 *p*H Meter

A-2.6 Pipette

A-2.7 Measuring Cylinder

A-3 REAGENTS

A-3.1 Water

A-3.2 Ferric Chloride

A-3.3 Natural Punica Dye (*Anar ka Chilka*)

A-3.4 Synthetic Yellow Dye

All reagents should be of analytical grade (AR).

A-4 PROCEDURE

Yellow dye samples (2-5 mg) are dissolved in water (10 ml) 2 ml solution of freshly prepared ferric chloride added and the mixture is shaken. Greenish blue colour develops in the solution having natural punica dye while the synthetic reactive yellow dye remains unchanged in colour.

A-5 CONCLUSION

The clear difference in colour identifies the natural punica dye and synthetic dye very distinctively as shown in Fig. 2.

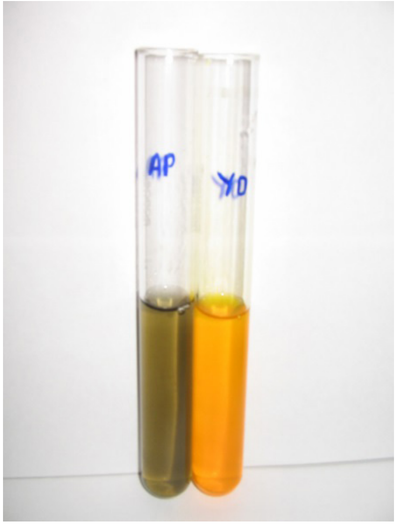


FIG. 2 CHANGE IN COLOUR FOR PUNICA DYE

ANNEX B

(*Clauses* 1, 5.2 *and* 5.3)

CHROMATOGRAPHIC ANALYSIS — THIN LAYER CHROMATOGRAPHY

B-1 PRINCIPLE

Thin layer chromatography is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). Natural punica dyes and synthetic yellow dyes are compared on TLC.

B-2 APPARATUS

B-2.1 Silica Coated TLC Plates

B-2.2 TLC Capillary

B-2.3 TLC Elution Chamber

B-2.4 TLC Iodine Chamber

B-2.5 Electronic Balance — Resolution at 0.001 g.

B-2.6 Spatula

B-2.7 Sonicator — To be set at 45 ± 2°C.

B-2.8 Measuring Cylinder

B-3 REAGENTS

B-3.1 Natural Punica Dye

B-3.2 Synthetic Reactive Yellow Dye

B-3.3 Ethyl Acetate

B-3.4 Hexane

B-3.5 Dichloromethane

B-3.6 Silica Gel (TLC Grade)

All reagents should be of analytical grade (AR).

B-4 PROCEDURE

Punica dye sample is prepared (0.01 g of *Anar ka Chilka* sample in 20 ml of solvent) by dissolved in solvents, such as ethyl acetate or dichloromethane. The prepared sample is agitated in sonicator, a mechanical agitating device used for dissolution of *Anar ka Chilka* dye molecules) for 5 min for complete dissolution of the punica samples. Similarly, synthetic yellow dye solution was prepared. The solution is then spotted on silica gel coated plate for elution. TLC plates were eluted using 20 percent methanol and 80 percent ethyl acetate (EtOAc) as suitable eluent system for yellow dyes. The spots were visualized in visible light as well as in iodine chamber. The possible constituents of the extracts are identified by comparing TLC data, that is, colour of the spot and R_f values of natural punica and synthetic yellow dye.

B-5 OBSERVATION

R_f on the TLC Plate	In Synthetic Reactive Yellow 15 (RY)	In Natural Punica PG	Comment (Describing Difference with Reason)
20 percent MeOH and 80 percent EtOAc	R_f 0.80	R_f 0.20	The polarity of the two dyes are distinctly different, punica being fairly polar dye

B-6 CONCLUSION

Color of the spot and R_f values of natural punica (PG) and reactive yellow (RY) are very different, punica being fairly polar dye as shown in the Fig. 3.

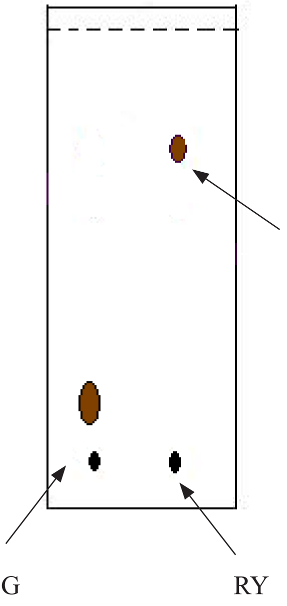


FIG. 3 TLC OF THE TWO DYES: NATURALPUNICA (PG) AND REACTIVE YELLOW (RY) DYE

ANNEX C

(*Clauses* 1, 5.2 *and* 5.3)

IDENTIFICATION OF NATURAL PUNICA DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD

C-1 PRINCIPLE

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. UV detector may be selected and set to optimal detection setting and develop a separation assay. The parameters of this assay shall be made to be such that a clean peak of the both the samples are observed from the chromatograph.

C-2 APPARATUS

C-2.1 HPLC Machine

C-2.2 PDA Detector

C-2.3 C-18 Reverse Phase Column (RPC-C18)

C-2.4 Electronic Balance — Resolution at 0.001g

C-2.5 Binary Pump System

C-2.6 Measuring Cylinder

C-2.7 Spatula

C-2.8 Micro Syringe

C-3 REAGENTS

C-3.1 Methanol

C-3.2 De-ionized Water

C-3.3 Natural Punica Dye

NOTE — All solvents used should be HPLC grade only.

C-4 PROCEDURE

The punica dye sample is weighed (0.1 g) in 1 000 ml of methanol and 10 µl of the prepared solution is injected to the C-18 reverse phase column and eluted through column. The base line shows response within a run of 15 min for natural punica as shown in Fig. 4. The parameters of this assay are made to be such that a clean peak of the both the samples are observed from the chromatograph.

The experimental conditions are as following:

a) Eluent: Methanol: Deionised water (97:03);
b) Stationary phase: Column C18, 150 × 4.6 mm; 5 micron;
c) Flow rate: 1.0 ml/min;
d) Detection wavelengths: 255 nm (band width 16 nm); and
e) Pump Pressure: 15 MPa.

Clear observation can be made from the punica dye sample as can be seen in the chromatographs given in Fig 4. Natural punica has main peak at 1.5 min and a characteristic peak at 4.0 min.

C-5 OBSERVATION

Peak Reading Received at	In Natural *Punica*	Comment (Describing Difference with Reason)
255 nm	1.5 mins main peak, small peaks at 4.0 min	One main peak is at 1.5 min in natural punica has other characteristic peak at 4.0 min

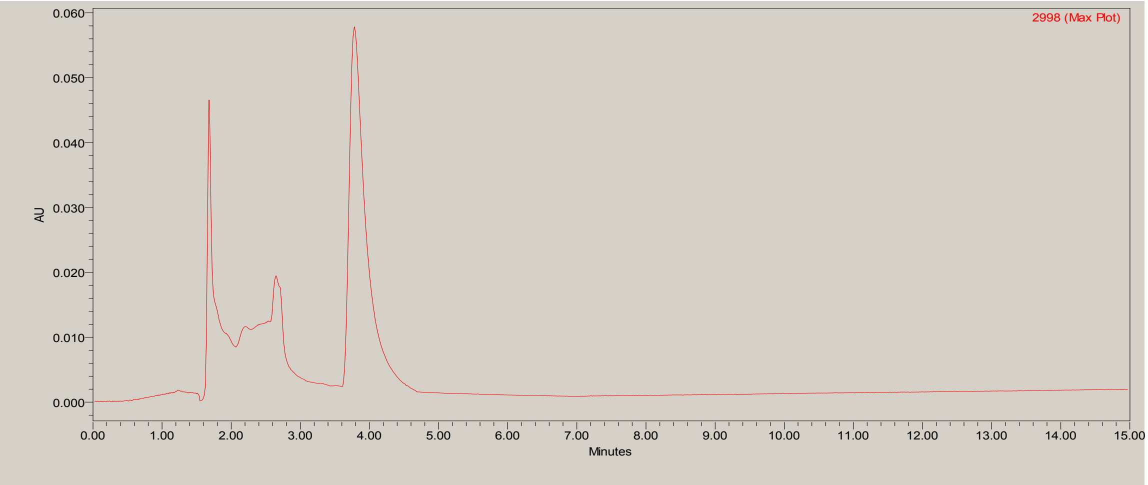


FIG. 4 CHROMATOGRAPH OF NATURAL PUNICA DYE

C-6 CONCLUSION

Natural punica shows clear differences in their characteristic peaks and their retention times and their peak heights. Natural punica will always be showing two equivalent peaks. This is a mark of identification for natural punica and synthetic dye.

ANNEX D

(*Clauses* 1, 5.2 and 5.3)

IDENTIFICATION OF NATURAL PUNICA DYE BY UV –VISIBLE SPECTROSCOPIC METHOD

D-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength.

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer’s law states that:

$$A = \varepsilon bc$$

Where, ε is a constant of proportionality, called the absorbtivity.

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. Thus, the absorption spectra (optical density) of a particular dye at a particular wavelength is maximum.

D-2 APPARATUS

D-2.1 Ultraviolet — Visible spectrophotometer machine.

D-2.2 Quartz Sample Tube (Cuvette) — Having 1.00 cm light path.

D-2.3 Electronic Balance — Resolution at 0.001g

D-2.4 Spatula

D-2.5 Whatmann Filter Paper Grade 42

D-3 REAGENT

D-3.1 Natural Punica Dye

D-3.2 Methanol

D-3.3 Dichloromethane

NOTE — All reagents shall be of analytical purity.

D-4 PROCEDURE

The two yellow dyes were weighed separately (0.1g) in 1 000 ml dichloromethane and scanned through UV-Visible spectrometer. For visible spectrum this solution is used and for UV spectrum the solution is further diluted 5 times. Identification of the dye by this method involves an empirical comparison of the details of the spectrum that is, lamda maxima (optical density) of natural punica and the characteristic peaks are matched as shown in Fig. 5. The ultraviolet (UV) region scanned is normally from 200 to 400 nm and the visible portion is from 400 to 800 nm.

D-5 OBSERVATION

Peak Reading Received at (nm)	In Natural *Punica*	Comment (Describing Difference with Reason)
	227 nm	Natural punica dye shows three prominent peaks as seen in the Fig. 4
	306 nm	
	364 nm	
	410 nm	

D-6 CONCLUSION

Lamda maxima (optical density) of natural punica is characteristic and different from the peak in visible spectrum is 410 nm.

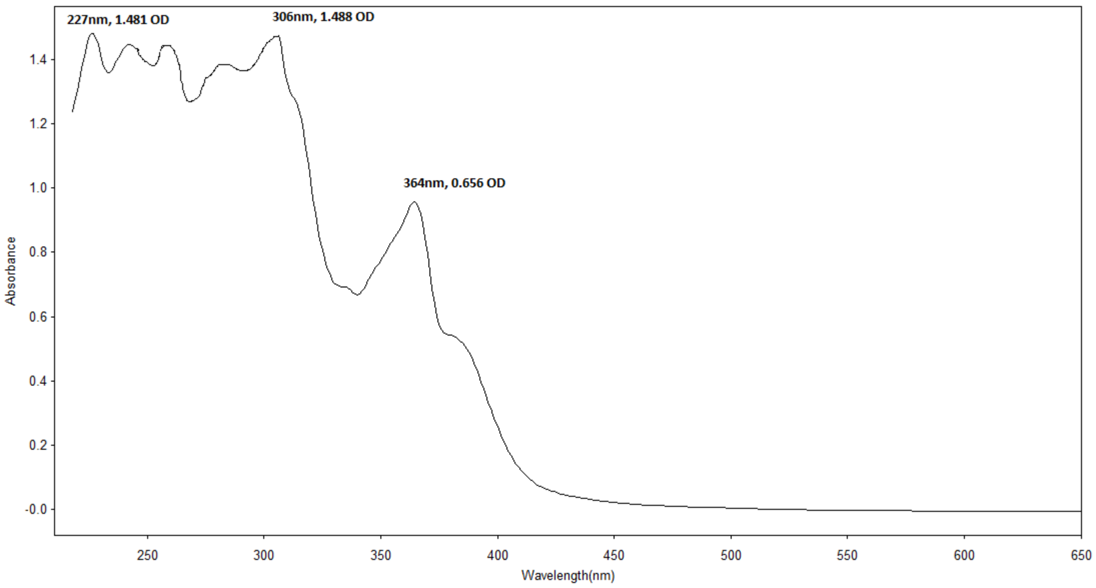


FIG. 5 UV SPECTRUM OF *PUNICA*

ANNEX E

(*Clause* 1 *and* 5.2)

EXTRACTION OF NATURAL PUNICA DYE FROM DYED SWATCHES

E-1 PRINCIPLE

The solvent (in soxhlet) extraction of dyed samples is necessary to release the dyes from the dyed fabric samples.

E-2 PROCEDURE

The solvent (in soxhlet) extraction of dyed samples is necessary to release the dyes from the dyed cotton samples. Cotton samples 50 g dyed from natural punica dye is cut into small pieces and packed in a filter paper as a filter paper column. The packed filter paper column is put for soxhlet extraction for 6-8 h with methanol as solvent for extraction. Temperature is set at 60°C till all the colour is discharged. Remove the solvent in a vacuum rotatory evaporator to dryness. The sample is now ready for used in chromatographic and spectroscopic analysis as per the methods described above.

ANNEX F

(*Foreword*)

COMMITTEE COMPOSITION

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

Organization	*Representative(s)*
Department for Jute and Fibre Technology Institute of Jute Technology University of Calcutta	Prof A. K. Samanta (***Chairman***)
Ahmedabad Textile Industry's Research Association, Ahmedabad	Shri C. R. Prayag Ms Bipasha Maiti (*Alternate*)
Ama Herbals, Lucknow	Shri Y. A. Shah
Atul Ltd (Colors Division), Atul	Dr Ashok Athalye Shri Arindam Chakraborty (*Alternate*)
Archroma India Pvt Limited, Mumbai	Shri Rajesh Ramamurthy Shri Ashim Ghosh (*Alternate*)
Central Institute for Research on Cotton Technology, Mumbai	Dr Sujata Saxena Dr A. S. M. Raja (*Alternate*)
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Indian Institute of Technology, Kanpur	Prof Raj Ganesh S. Pala
Indian Jute Industries Research Association Kolkata	Dr S. K. Chakrabarti Shri Sandip Basu (*Alternate*)
Jaysynth Dye-Chem, Mumbai	Shri S. C. Sharma Shri P. M. Reshamwala (*Alternate*)
National Peroxide Limited, Mumbai	Shri K. H. Deshpande Shri V. N. Joshi (*Alternate*)
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Wool Research Association, Thane	Smt Smita Bait Shri Chetan Mahajan (*Alternate*)
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